International Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines: State of the Science and the Path Forward

William H. Natcher Conference Center, National Institutes of Health
Bethesda, MD, USA
November 28-29, 2012

Organized by International Cooperation on Alternative Test Method (ICATM) Members:

NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
JaCVAM	Japanese Center for the Validation of Alternative Methods
KoCVAM	Korean Center for the Validation of Alternative Methods
Health Canada	

Co-Sponsored by:
National Institute of Environmental Health Sciences
National Toxicology Program
International Alliance for Biological Standardization (IABS) *(pending)*

Overview

Introduction

Pertussis is a highly contagious disease caused by the bacterium *Bordetella pertussis. In infants a pertussis infection is characterized by* uncontrollable, violent coughing accompanied by a deep "whooping" sound when the patient tries to take a breath. Pertussis was formerly one of the most common childhood diseases of the early 20th century and a major cause of childhood mortality in the United States. In westernized countries, the incidence of pertussis has been reduced by more than 80% since the advent of a whole-cell vaccine in the 1940s. Public concern regarding some common side effects (e.g., fever, swelling at injection site) and serious events that occurred at very low frequency in temporal association with the use of whole-cell pertussis vaccines, led to the successful development of an acellular pertussis (aP) vaccine in the early 1980s. These new generation vaccines contain different combinations of the putative protective antigens of *B. pertussis* bacteria (e.g., inactivated pertussis toxin (PTx/d), pertactin, and fimbriae), and are less reactogenic than whole-cell vaccines. However, since the 1980s there has been an increase in the number of reported cases of pertussis, especially among 10- to 19-year-olds and infants younger than 6 months of age. This has led to the recent recommendation by the U.S. Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices to expand the pertussis vaccination recommendation to include all adults, 19 and older who have not previously received a TdaP (tetanus, diphtheria, and pertussis combined) vaccination, including those aged 65 and older.

Vaccine Lot Release Testing

Regulatory authorities require safety, potency, and purity testing prior to the release of each production lot of pertussis or pertussis-containing vaccines. The murine histamine sensitization test (HIST) is a key safety test used to monitor residual levels of pertussis toxin in acellular pertussis vaccines. This test is performed to ensure that pertussis toxin has been effectively inactivated before release of vaccines. However, such testing may involve large numbers of mice, some of which can experience significant unrelieved pain and distress. In addition, the HIST has technical challenges requiring frequent re-testing, thereby increasing vaccine testing expense. An international workshop organized in 2010 by NICEATM, ICCVAM, and their international partners identified the HIST as a priority for future research, development, and validation of alternative test methods that could further reduce, refine (enhance animal well-being and lessen or avoid pain and distress), or replace animal use for acellular pertussis vaccine safety testing.[1]

Recent International Meetings

Recently, two international workshops reviewed currently available alternative *in vitro* assays to the HIST and discussed a path forward to achieve their validation and adoption[2,3]. The Workshop on Animal-Free Detection of Pertussis Toxin (PTx) in Vaccines – Alternatives to HIST was held on June 9 and 10, 2011, at the Paul Ehrlich Institute, Germany. It brought together interested stakeholders working on HIST alternative assays, defined regulatory acceptance criteria that would be required for any such alternative methods and established the International Working Group for Alternatives to HIST.

The Alternative Safety Testing Strategies for Acellular Vaccines Workshop was held as a satellite meeting to the 8th World Congress on Alternatives and Animal Use in the Life Sciences on August 21, 2011 in Montreal, Canada. This workshop further discussed and clarified regulatory agency requirements to achieve the acceptance of alternative methods to the HIST. Participants also discussed strategies for the adoption of international regulatory requirements based on the concept of consistency of manufacture. In addition, the workshop addressed the requirements and procedures for assay validation and comparability studies to be performed on pertussis toxin-spiked vaccine samples. Participants agreed that a study using spiked vaccines to compare the sensitivities of the HIST and *in vitro* assays would be important. It was also agreed that the direct correlation between the *in vivo* and *in vitro* assays was not required and attempting to correlate the data could be detrimental to the study outcome.

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1 International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions, Bethesda, MD, USA September 14-16, 2010

2 Workshop on Animal-Free Detection of PTx in Vaccines – Alternatives to HIST, Langen, Germany, June 9-10, 2011

3 Alternative Safety Testing Strategies for Acellular Pertussis Vaccines (8th World Congress Satellite meeting), Montreal, Canada, August 21, 2011

International Working Group for Alternatives to HIST

The International Working Group for Alternatives to HIST, composed of regulatory and industry participants, was established to facilitate the evaluation of alternative *in vitro* methods for PTx measurement. The group coordinated the acquisition and distribution of acellular pertussis vaccine samples from manufacturers to research laboratories for generation of data on *in vitro* methods on vaccines spiked with a known amount of PTx. A total of seven vaccines from three manufacturers (GlaxoSmithKline, Sanofi Pasteur, Statens Serum Institute) were provided to 12 international laboratories. The working group has been instrumental in determining an appropriate method for spiking vaccines with a known quantity of pertussis toxin, as well as establishing a framework for the development, evaluation, and validation of alternative methods. Data from various alternative assays using commonly spiked vaccines, as well as on the impact that adjuvants have on the assay performance will be presented at our workshop and will form the basis for selection of the *in vitro* method or methods that will be assessed in the next international collaborative study.

Several *in vitro* assays have been developed, or are currently under development, with the aim of finding an alternative method to HIST for monitoring residual PTx activity in acellular pertussis vaccines. The following methods will be evaluated and may be used to generate data to be presented at the upcoming workshop:

1. Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin
2. Enzymatic assay: monitors the residual ADP-ribosylation of the pertussis toxin/toxoid
3. Cell-based assays: monitor the generation of cAMP or decrease in cellular ATP, following exposure to pertussis toxin
4. Genetic assays: determine potential genomic markers of pertussis toxin activity

This workshop will provide a forum to discuss and review the *in vitro* protocols and available data from the international pertussis toxin spiked-vaccine study and will suggest future collaborative projects using prepared materials. The workshop will also review recent advances and innovations in science and technology that can be applied to new methods and approaches for acellular pertussis vaccine safety testing that are more humane, use fewer or no animals, and may provide greater accuracy, precision, and efficiency. Finally, the workshop will address the path toward global acceptance, validation, and implementation of scientifically valid alternative methods for acellular pertussis vaccines.

Draft Workshop Objectives

1. Review the usefulness and limitations for alternative *in vitro* test methods proposed to replace the current *in vivo* HIST
    - Review of *in vitro* protocols and data generated by participants of the pertussis toxin spiked vaccine study
        a. Use of a common set of vaccines, pertussis toxin (reference standard) and protocol for spiking
        b. *In vitro* assays tested
            i. Biochemical assays
                1. Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin
                2. Enzymatic assay: monitors the residual ADP-ribosylation of the pertussis toxin/toxoid
            ii. Cell-based assays
                1. Human cells (PBMC) measuring ATP reduction
                2. Rat vascular smooth muscle cell line (A10) measuring cAMP
                3. CHO cell line (morphological/growth changes)
2. Discuss the application of these *in vitro* assays for monitoring consistency of vaccine manufacture as alternatives to the HIST
3. Establish a framework for international collaboration to achieve the adoption of *in vitro* assay(s) for acellular pertussis vaccine testing
4. Lay the groundwork for regulatory acceptance of a harmonized approach to *in vitro* assays as alternatives to the HIST

Draft Agenda

— Day 1 —
Wednesday, November 28, 2012

7:30-8:00 **Registration and Poster Setup**

8:00 **Opening Session**

Co-chairs:
Juan Arciniega, DSc, Center for Biologics Evaluation and Research, U.S. FDA
Richard McFarland, PhD, MD, Center for Biologics Evaluation and Research, U.S. FDA

8:00-8:15 **Welcoming Remarks and Introduction to ICATM Organizations**

William Stokes, DVM, RADM, USPHS, National Institute of Environmental Health Sciences, NIH, Executive Director, ICCVAM

8:15-8:30 **Workshop Overview and Objectives**

Richard McFarland, PhD, MD, CBER, U.S. FDA

8:30-9:00 **Plenary Presentation: The Many Faces of Pertussis Toxin**

Nicholas Carbonetti, PhD, University of Maryland, USA

9:00-9:20 **Current Regulatory Requirements for Residual Pertussis Toxin Testing of Acellular Vaccines**

Sue Nelson, PhD, Sanofi Pasteur, Canada

9:20-9:40 **International Collaborative Study on Validation of an *In Vitro* Assay System as an Alternative to Current Histamine Sensitization Test for Acellular Pertussis Vaccines**

Dorothy Xing, PhD, National Institute for Biological Standards and Control, UK

9:40-10:00 **Overview of the International Working Group for Alternatives to HIST: Phase I**

Richard Isbrucker, PhD, Health Canada

10:00-10:20 **Break**

Session 1
Alternatives to HIST: Methods and Evaluations

10:20-12:20 **Session 1A**
Reports on Alternative Methods to HIST and Results using Pertussis Toxin-Spiked Vaccine Samples

Co-chairs:
Sue Nelson, PhD, Sanofi Pasteur, Canada
Marieke Hoonakker, MSc, Netherlands Vaccine Institute, Netherlands

10:20-12:20 **Presentations** *(20 min each)*

12:20-1:30 **Lunch and Poster Session**

1:30-2:30 **Poster Session (cont.) and Additional Presentations *(if required)***

2:30-5:00 **Session 1B**
Alternative *In Vitro* Methods to the Murine Histamine Sensitization Test

Co-chairs:
Richard McFarland, PhD, MD, CBER, U.S. FDA
Christina Bache, PhD, Paul-Ehrlich-Institut, Germany

- Roundtable discussion

— Day 2 —

Thursday, November 29, 2012

7:30-8:00 **Registration**

8:00-3:00 **Session 2**
The Path Forward: Gaps to Cross and Bridges to Build in the Road Towards the Adoption of Alternatives to HIST

8:00-8:15 **Opening Remarks**

Richard Isbrucker, PhD, Health Canada

8:15-9:00 **Plenary Presentation: What the CHO Cell Assay tells us about Pertussis Activity and the Pathophysiology of Whooping Cough**

Erik Hewlett, MD, University of Virginia, USA

9:00-10:30 **Session 2A**
CHO Cell Assay – Potential Use for Standardization and Alternative to HIST

Co-chairs:
Richard Isbrucker, PhD, Health Canada
Amélie Castiaux, PhD, GlaxoSmithKline, Belgium

- Roundtable discussion

10:30-11:00 **Break**

11:00-12:30 **Session 2B**
Issues with Pertussis Toxin Adsorption/Desorption

Co-chairs:
Dorothy Xing, PhD, NIBSC, UK
Juthika Menon, PhD, Sanofi Pasteur, Canada

- Roundtable discussion

12:30-1:30 **Lunch**

1:30-3:00 **Session 2C**
The Path Forward: Harmonizing the Adoption of Alternative Assays

Co-chairs:
Juan Arciniega, DSc, CBER, U.S. FDA
Richard Isbrucker, PhD, Health Canada

- Roundtable discussion

3:00-3:30 **Break**

3:30-5:00 **Session 3**
Next International Collaborative Validation Study on Alternative Assay(s) with Spiked Vaccines

Co-chairs:
Jean-Michel Chapsal, PhD, Sanofi Pasteur, France
Lev Sirota, PhD, CBER, U.S. FDA

- Roundtable discussion

5:00 **Closing Remarks and Adjournment**

William Stokes, DVM, RADM, USPHS, National Institute of Environmental Health Sciences, NIH, Executive Director, ICCVAM

5:45 **End of Meeting**